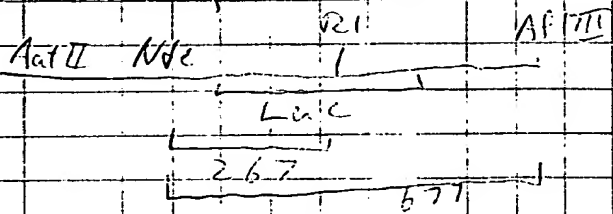
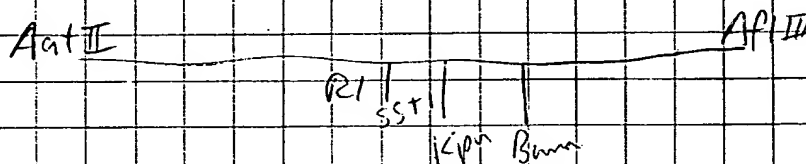


ag N — cut on at Nde I to see if all of linc is present even though Aat II is missing



Nde I is OK in buffer 4 (NEB) (P169)
 expect 677bp Nde I / Afl III band

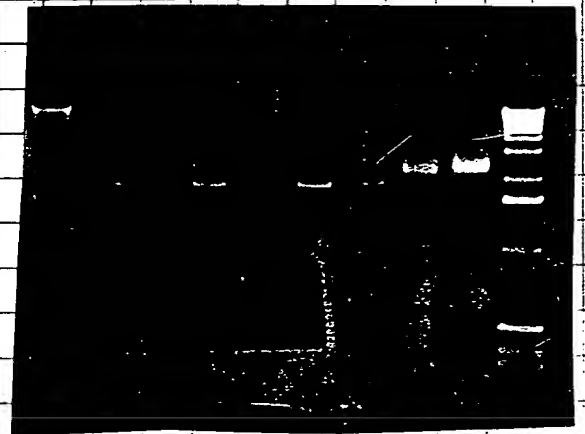
cut with Sst I, Kpn I or Bam HI to see if missing. R1 site is only a point mutation (and OK in NEB buffer 4)



(for cloning #20
 (tubes 2, 4, 6 below)
 see if Sst I, Kpn I or Bam
 can cut in MCS

Tube #	1	2	3	4	5	6	7	8	
A #1	5	5	5	5	5				✓
blue cloning									
(missing R1)		5	5	5	5				✓
missing Aat II								5	✓
Aat II	0.1								✓
R1 III	0.3								✓
I	1	1							
A I			1	1					
Bam HI					1	1			
R1						1	1		
1/20	11.6								✓
Buffer 4	2								✓
Result	20 µl								

Sst I Kpn I Bam HI



37°C 2 hr

G A A T T C G A G C T C

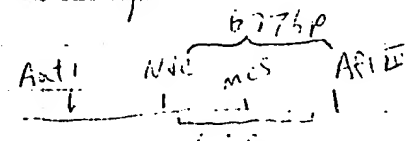
R1 Sst I

Sst I cuts but R1 did not (P106)

so mutation is no more than 1 bp downstream from the R1 site and may even be a

point mutation

Nde may give the 677 bp fragment expected but multiple bands are seen



To Page No. _____

ssed & Understood by me,

earuara Polaro

Date

2/16/95

Invented by

R cord d by

Date

1/13/95